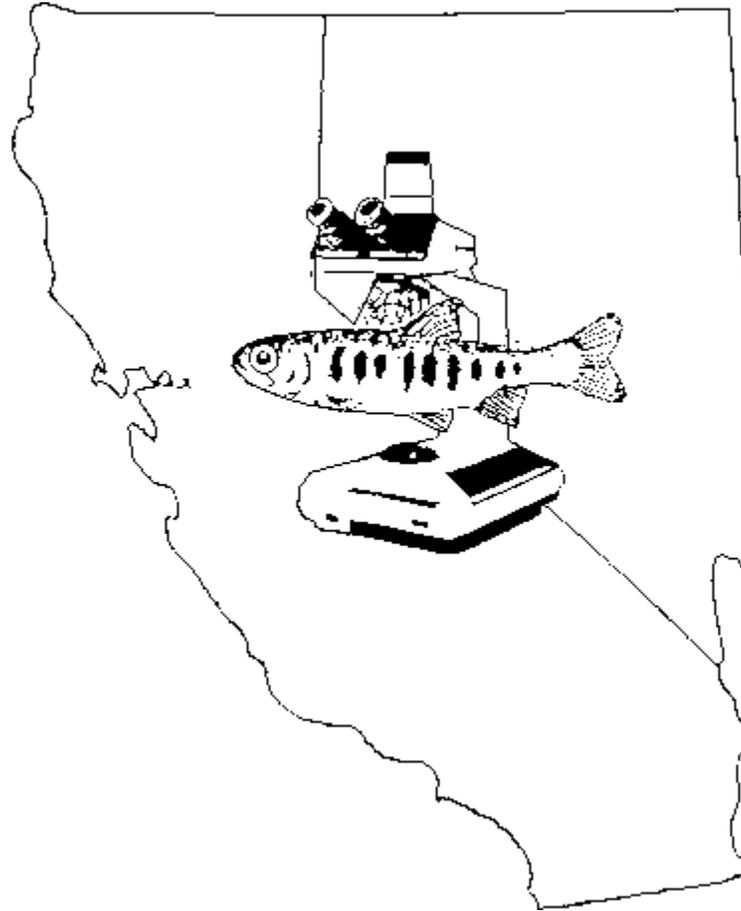


FY 2003 Investigational Report:

Health and Physiological Assessment of VAMP Release Groups – 2003



Ken Nichols* and J. Scott Foott
US Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, CA

August 2003

* Direct correspondence:
CA-NV Fish Health Center
24411 Coleman Fish Hatchery Rd
Anderson, CA 96007
(530) 365-4271 Fax: (530) 365-7150
Kenneth_Nichols@fws.gov

Summary: The incidence of clinical Proliferative Kidney Disease was notably higher in smolts used for the second set of releases and could reduce their survival in comparison to cohorts in the first releases. No biologically significant differences in smolt development or stress response were detected among fish from the different release sites. Plasma ion balance was disturbed in fish held in live boxes for up to 4 hours post release but returned to normal after 24 hours

Methods

Merced River Hatchery (MRH) Chinook salmon smolts from both the first (4/21 – 4/25/03) and second Vernalis Adaptive Management Plan (VAMP) study releases (4/28 – 5/02/03) were examined for general health and post-release stress responses. A total of 224 fish were examined from the 6 release groups following transport to release sites at Durham Ferry, Mossdale and Jersey Point. Both internal and external abnormalities were recorded for each smolt. The following tissues were collected from a subset of fish in each release group: kidney tissue for histological detection of *Tetracapsula bryosalmonae* infection (the parasite causing Proliferative Kidney Disease (PKD)), gill tissue for Na⁺-K⁺-ATPase (ATPase) activity assays, and plasma for stress indicator assays (chloride, sodium, lactate, glucose, total protein and cortisol). In order to examine stress recovery dynamics, blood chemistry samples were taken from fish directly out of the transport truck (0 hours), and after being held in river live boxes (3150 cm³ volume, 5 fish/box) for 2 and 4 hours post-release. An additional blood sample was obtained at 24 hours post-release from the 5/02/03 Jersey Point and both Durham Ferry release groups. A general health inspection for viral, *Renibacterium salmoninarum* (Bacterial Kidney Disease agent) and systemic bacterial infection was performed on sixty salmon from the 4/22/03 Mossdale release group.

Results and discussion

No viral pathogens or *R. salmoninarum* were detected in the 60 fish sample. Low colony counts of bacteria in the *Aeromonas* / *Pseudomonas* and *Staphylococcus* / *Micrococcus* groups were isolated from 18 of 60 fish (30%). Sub-clinical infections of these aquatic bacteria are common in fish as they are part of the bacterial flora of the skin and gastrointestinal tract. We do not consider these isolations as significant health risks.

Tetracapsula bryosalmonae was detected in 63% (30/48) of kidneys, and 21% (10/48) showed severe inflammation due to the infection (Table 1). Gross clinical signs of PKD (swollen kidney and/or spleen) were noted in 11% (25 / 222) of smolts examined and tended to be more prevalent in the second set of releases (P<0.001, z-test, Table 1). Proliferative kidney disease has been observed in MRH Chinook smolts for many years, and incidence in VAMP release groups has ranged from 4% to 100% in the last 4 years (Table 2). This progressive disease can reduce a fish's performance due to the associated kidney dysfunction and anemia. Smolts in the first release group may have higher survival than cohorts in the second release due to their earlier (reduced) stage of PKD.

Table 1. Prevalence of *Tetracapsula bryosalmonae* clinical signs and infection in 2003 VAMP release groups. Swollen kidney (kd) and spleen (Sp) assessed by gross observation of the organs during necropsy, and *Tb* infection and kidney (kd) lesion assessed by histology.

Sample site	Release date	Swollen kd	Swollen sp	<i>Tb</i> infection	Kd lesion
Durham Ferry	4/21/03	1/71 (1%)	1/71 (1%)	7/12 (58%)	1/12 (8%)
Mossdale	4/22/03	2/30 (7%)	3/30 (10%)	5/6 (83%)	2/6 (33%)
Jersey Point	4/25/03	0/29 (0%)	0/29 (0%)	3/6 (50%)	3/6 (50%)
Durham Ferry	4/28/03	8/39 (21%)	6/39 (15%)	4/6 (67%)	1/6 (17%)
Mossdale	4/29/03	3/13 (23%)	3/13 (23%)	7/12 (58%)	1/12 (8%)
Jersey Point	5/02/03	8/40 (20%)	8/40 (20%)	4/6 (67%)	2/6 (33%)

Table 2. Prevalence of *Tetracapsula bryosalmonae* detected in Merced River Hatchery Chinook Salmon smolts 1996-2003. All samples were taken from VAMP (and precursor project) release groups. Fish were assayed by histopathological examination of posterior kidney by the CA-NV Fish Health Center.

Year	Sample Date(s)	Prevalence
1996	5/01	5/8 (63%)
1997	5/01	0/10 (0%)
1998	4/17	0/6 (0%)
1999	4/20	0/6 (0%)
2000	4/18 – 5/02	2/45 (4%)
2001	5/01 – 5/12	34/34 (100%)
2002	4/19 – 5/01	92/201 (46%)
2003	4/21 – 5/02	30/48 (63%)

All sample groups demonstrated similar levels of smolt development as demonstrated by gill ATPase activity ($P=0.080$, ANOVA on Ranks, $n = 64$). The overall median gill ATPase activity was 7.1 mmol ADP / mg protein / hour. The observed ATPase levels were consistent with fish undergoing smoltification.

There were few consistent patterns or site-specific differences in blood chemistry values observed between the different release groups (Table 3). We selected the time zero samples (collected directly from the transport truck) as reference data to examine recovery dynamics; however, we realize that these values do not represent a true “baseline”. It would be necessary to sample fish that had not been subjected to stress within 24 or more hours to determine a true “baseline”. It would appear that live box confinement induced additional stress on the transported fish as indicators of stress (cortisol, glucose, lactate) tended to remain altered throughout the sampling period (up to 24hrs). Blood electrolyte loss can continue up to 4 hours following stress and a loss of 30% or more can be lethal (McDonald and Milligan 1997). Plasma chloride was below normal in 4 of 5 groups at 4 hours post-release, but chloride did return to a normal range in the 24 hours samples. There were no biologically significant shifts in plasma protein levels detected in any group. The accuracy of the sodium data is suspect given the many high values measured in the flame photometer assay. Trend data for sodium regulation showed little loss of ion control with the exception of the second Jersey Point release group that had lower than “time zero” values through the 24hr sample.

It should be noted that comparison of the release groups is complicated by differences in transport time and handling prior to entry into the live boxes. The “noise”

created by these site variations may hide some trends in blood chemistry values that signal survival differences in the release groups. There may also be problems with extrapolating blood chemistry values of smolts held in live boxes to those released into the river. In the future, better data could be obtained by sampling both unstressed smolts and those held for 24-48 hours in live boxes at the hatchery for baseline blood chemistry data.

We feel that the fish health study design could be changed to better describe the release group's potential performance. Short (within year) and long term (year-to-year) PKD status of the study groups is one major health factor we have identified in MRH smolts. While blood chemistry profiles can give us an indication of a group's potential performance, sample conditions must be consistent for valid comparison between groups. We recommend continued health monitoring for comparison of short and long term trends (particularly PKD), and standardized treatment of sample groups prior to any blood collection.

Acknowledgements

We wish to acknowledge Ron Stone with the CA-NV Fish Health Center for covering the 4/25 Jersey Point sample (night shift). Thanks also to Paul Cadrett with the Stockton Fish and Wildlife Office for coordination and vehicle access assistance.

Table 3. Blood plasma chemistry values for VAMP 2003 release groups sampled at the release site. Smolts were sampled immediately after transport (0 hours) and from live boxes held in the river at 2, 4 and 24 hours after release. Data is presented as Median (n) and 10th to 90th percentile data range. Values significantly different than the 0 hour sample at each site denoted with asterisk (P<0.050, Rank Sum Test).

Assay ¹	Sample group	Release date	Hours after transport				10%-90% of range
			0	2	4	24	
Chloride (mEq/L)	Durham Ferry	4/21/03	114 (15)	101* (15)	104* (16)	107 (15)	91-127
	Mossdale	4/22/03	90.5 (8)	97 (8)	112 (8)	ND	
	Jersey Point	4/25/03	110 (7)	102* (8)	102.5(8)	ND	
	Durham Ferry	4/28/03	113 (7)	100* (9)	104* (9)	115 (10)	
	Mossdale	4/29/03	111.5 (10)	ND	ND	ND	
	Jersey Point	5/02/03	120.5 (8)	109* (8)	109* (8)	119 (8)	
Sodium (mmol/L)	Durham Ferry	4/21/03	181 (7)	194 (8)	214 (10)	184 (13)	151-213
	Mossdale	4/22/03	176 (7)	162.5 (2)	168 (3)	ND	
	Jersey Point	4/25/03	175 (7)	160 (7)	154 (7)	ND	
	Durham Ferry	4/28/03	172.5 (8)	162* (10)	159 (9)	170 (10)	
	Mossdale	4/29/03	173.5 (10)	ND	ND	ND	
	Jersey Point	5/02/03	173 (7)	156 (7)	149* (3)	158* (6)	
Lactate (mg/dl)	Durham Ferry	4/21/03	40 (8)	64* (8)	58.5* (8)	58.5* (8)	16-71
	Mossdale	4/22/03	33 (7)	67* (8)	50* (7)	ND	
	Jersey Point	4/25/03	69 (8)	28* (8)	38* (8)	ND	
	Durham Ferry	4/28/03	22 (7)	19 (10)	25.5 (10)	11.5 (10)	
	Mossdale	4/29/03	14.5 (10)	ND	ND	ND	
	Jersey Point	5/02/03	70.5 (8)	42* (8)	39.5* (8)	31.6* (8)	
Glucose (mg/dl)	Durham Ferry	4/21/03	77.5 (16)	98 (16)	122* (16)	87 (16)	62-163
	Mossdale	4/22/03	98.5 (8)	134 (7)	129 (8)	ND	
	Jersey Point	4/25/03	130.5 (8)	86.5 (8)	119.5 (8)	ND	
	Durham Ferry	4/28/03	79 (7)	76 (10)	83 (9)	71.5 (10)	
	Mossdale	4/29/03	76 (10)	ND	ND	ND	
	Jersey Point	5/02/03	87 (8)	122 (8)	120 (7)	91 (8)	
Total Protein (g/dl)	Durham Ferry	4/21/03	3.0 (15)	3.5 (16)	3.5* (16)	3.1 (16)	2.7-4.0
	Mossdale	4/22/03	3.3 (7)	3.9 (8)	3.9 (8)	ND	
	Jersey Point	4/25/03	3.6 (8)	4.0 (8)	3.3 (8)	ND	
	Durham Ferry	4/28/03	2.8 (7)	3.0 (10)	3.3* (9)	3.2 (10)	
	Mossdale	4/29/03	3.1 (10)	ND	ND	ND	
	Jersey Point	5/02/03	3.1 (8)	3.3* (8)	3.2 (7)	3.2 (8)	
Cortisol (ng/ml)	Durham Ferry	4/21/03	39.5 (4)	36.5 (4)	41.5 (4)	57.5 (4)	33-68
	Mossdale	4/22/03	41.5 (4)	42 (4)	58 (4)	ND	
	Jersey Point	4/25/03	48.5 (4)	39 (3)	58.5 (2)	ND	
	Durham Ferry	4/28/03	35 (3)	36 (4)	61.5 (4)	46 (4)	
	Mossdale	4/29/03	41 (4)	ND	ND	ND	
	Jersey Point	5/02/03	60 (3)	46.5 (4)	54 (4)	59 (4)	

¹ Assay Methods (all colorimetric methods modified for 96 well microplate):

Chloride determined by colorimetric method using Raichem kit #85133 (San Diego, CA)

Sodium determined by flame photometer (Jenway, Essex, England)

Lactate determined by Trinder colorimetric method using Randox product #LC2389 (San Diego, CA)

Glucose determined by Trinder colorimetric method using Raichem kit #80038 (San Diego, CA)

Total Protein determined by biuret colorimetric method using Sigma Diagnostics #541-2 (St. Louis, MO)

Cortisol determined by ELISA kit produced by Neogen Corporation (#402710, Lexington, KY)

References

McCormick, S.D. and H.A. Bern. 1989. In vitro stimulation of Na⁺-K⁺-ATPase activity and ouabain binding by cortisol in Coho salmon gill. *American Journal of Physiology*. 256: R707-R715.

McDonald, G. and L. Milligan. 1997. Ionic, osmotic and acid-base regulation in stress. Pages 119-144. In G.K. Iwama, A.D. Pickering, J.P. Sumpter and C.B. Schreck (eds.). *Fish Stress and Health in Aquaculture*. Cambridge University Press, Cambridge, UK.